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Immune senescence and cancer in elderly patients: Results from an exploratory study☆

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ABSTRACT

Background: The challenge of immune senescence has never been addressed in elderly cancer patients. This study compares the thymic output and peripheral blood telomere length in ≥70 year old cancer patients.
Patients and methods: Fifty-two elderly cancer patients and 39 age-matched controls without personal history of cancer were enrolled. All patients underwent a Comprehensive Geriatric Assessment (CGA), from which a multidimensional prognostic index (MPI) score was calculated. Peripheral blood samples were studied for naïve and recent thymic emigrant (RTE) $CD4^+$ and $CD8^+$ cells by flow cytometry. T-cell receptor rearrangement excision circle (TREC) levels, telomere length and telomerase activity in peripheral blood cells were quantified by real-time PCR.
Results: The percentages of $CD8^+$ naïve and $CD8^+$ RTE cells and TREC levels were significantly lower in cancer patients than in controls ($p = 0.003$, $p = 0.004$, $p = 0.031$, respectively). Telomere lengths in peripheral blood cells were significantly shorter in cancer patients than in controls ($p = 0.046$) and did not correlate with age in patients, whereas it did in controls ($r = -0.354$, $p = 0.031$). Short telomere (≤median)/low TREC (≤median) profile was associated with higher risk of cancer (OR = 3.68 [95% CI 1.22–11.11]; $p = 0.021$). Neither unfitness on CGA nor MPI score were significantly related to thymic output or telomere length in either group.
Conclusions: Immune senescence is significantly worse in elderly cancer patients than in age-matched controls. The low thymic output and the shorter telomeres in peripheral blood cells of cancer patients may reflect a pre-existing condition which facilitates the onset of malignancies in elderly people.

1. Introduction

People over 65 years old are the fastest-growing age bracket in the population and will account for an estimated 20% of Americans and 25% of Europeans by the year 2030 (Fries, 2003). The incidence of malignancies increases with age, so the number of cancers in the elderly is expected to increase significantly in years to come (American Cancer Society, 2012). Several studies have shown that elderly patients are less likely to be treated according to guidelines, and their under-treatment may be detrimental to both survival and quality of life (Bouchardy et al., 2003; Dale, 2003; Ng et al., 2005; Sargent et al., 2001). Elderly cancer patients may benefit from chemotherapy just as much as younger adults, but at a higher risk of hematological toxicity (Hurria et al., 2012; Muss et al., 2005, 2007). A better understanding of the physiological and functional changes that occur with aging will enable us to improve strategies for treating elderly cancer patients. Since the aging process coincides with a gradual decline in the functional reserve of multiple organ systems (Balducci, 2003) the search for laboratory markers of biological aging and organ reserve should be a priority of clinical research in the field of geriatric oncology.

Over a lifetime, the immune system undergoes a profound remodeling process with major impact on health and survival (Fulop et al., 2010; Grubeck-Loebenstein et al., 2009). Thymic involution and diminished

Abbreviations: CGA, comprehensive geriatric assessment; MPI, multidimensional prognostic index; RTE, recent thymic emigrant; TREC, T-cell receptor rearrangement excision circle; RU, relative units; ADL, Activities of Daily Living; IADL, Instrumental Activities of Daily Living; SPMSQ, Short Portable Mental Status Questionnaire; MMSE, Mini Mental Status Examination; CIRS-CI, Cumulative Illness Rating Scale-Comorbidity Index; CIRS-SI, Cumulative Illness Rating Scale-Severity Index; GDS, Geriatric Depression Scale; MNA, Mini Nutritional Assessment; T/S, telomere/single-copy gene; PBMC, peripheral blood mononuclear cells; CMV, Cytomegalovirus.

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output of T lymphocytes are thought to be among the major factors contributing to the loss of immune function with age (Berzins et al., 1998). T cell output begins to decline exponentially from early in life, and at 75 years of age the immune repertoire appears to be severely impaired (Douek et al., 1998; Naylor et al., 2005). However, recent data suggest that the thymus may remain active even late in life, supplying functional T cells to the periphery (Mitchell et al., 2010; Nasi et al., 2006).

Measuring T cell receptor rearrangement excision circle (TREC) levels in peripheral blood lymphocytes has been suggested as a method for quantifying thymic output (De Rossi et al., 2002; Douek et al., 1998; Ometto et al., 2002; Zhang et al., 1999). TRECs are generated by T cell receptor gene rearrangement and maintained in thymic emigrant cells as DNA episomes. Because TRECs are not duplicated during mitosis, their concentration is diluted out with each cell division. The frequency of recent thymic emigrant (RTE) cells in peripheral blood, identified by the marker $CD31^+$ among $CD45RA^+$ naïve T cells (Kimmig et al., 2002), decreases with aging and correlates well with the decline in TREC levels (Junge et al., 2007; Kohler et al., 2005). Very little is known about the relationship between TRECs and cancer, especially in elderly patients. One study on head and neck cancer patients, including only a few ≥70 years old, showed that the age-associated decrease of TREC numbers and naïve T lymphocytes was significantly greater in cancer patients than in controls, suggesting altered lymphocyte homeostasis in the former (Kuss et al., 2005).

The immune system function depends largely on its capacity for extensive cell division and clonal lymphocyte expansion. Telomere length and its regulation by telomerase have attracted considerable attention, due to their potential roles in controlling cell replication (Blackburn et al., 2006). Telomeres are capping end structures of eukaryotic chromosomes essential for protecting chromosome integrity (Blasco, 2005). Telomeres are progressively shortened during each cell division due to end-replication problems of DNA polymerase; when a critical length is reached, the cell undergoes cycle arrest and apoptosis (Blasco, 2005). Permanent cell growth relies on telomere maintenance, and certain human cell subsets, as well as most cancer cells, have telomerase activity which enables telomere elongation (Dolcetti and De Rossi, 2012). Despite their telomerase activity, most tumor cells have shorter telomeres than the corresponding normal tissues, and there is a relationship between short telomeres and genetic instability (Garcia-Aranda et al., 2006; Rampazzo et al., 2010). Since telomere shortening reflects cell turnover and exposure to oxidative and inflammatory damage, which are crucial processes in biological aging, it has been suggested that telomere length serves as an indicator of aging (Aviv, 2006; Baird, 2006; Wong and Collins, 2003). Telomere shortening in peripheral blood cells has been associated with a number of chronic diseases, such as coronary heart disease, hypertension, dementia, obesity, insulin resistance, and osteoporosis. However, two clinical trials failed to confirm any relationship between telomere length and frailty syndrome in elderly non-cancer patients (Collerton et al., 2012; Woo et al., 2008).

Several studies have investigated the relationship between telomere length in peripheral blood cells and cancer risk. Although few manuscripts report that longer telomere length is a risk factor (e.g. Lan et al., 2013; Svenson et al., 2008), most of the studies indicate that short telomere lengths are associated with a higher cancer risk (Bojesen et al., 2013; Martinez-Delgado et al., 2012; Riegert-Johnson et al., 2012; Shao et al., 2007; Wu et al., 2003). To date, however, telomere length in peripheral blood cells has been measured in elderly non-cancer patients and younger cancer patients, but no data are available for elderly cancer patients. We present here the results of a prospective observational study providing the first description of immune senescence markers and frailty scores in elderly cancer patients and age-matched controls.

2. Material and methods

2.1. Study design and study population

Patients enrolled in this study were aged ≥70 years, with stages I–III breast or colorectal cancer, diagnosed during the previous 2 months and radically resected, consecutively admitted to the Medical Oncology Units from September 2010 to March 2012. Controls included patients ≥70 years old with no personal history of cancer, consecutively admitted to the Geriatric Clinic. For both groups, the exclusion criteria were: any hematological disorders, chronic diseases requiring immunosuppressive treatment, prior immunodeficiency, blood transfusion ≤4 weeks before blood sampling, active infectious diseases, extremely severe comorbidities suggesting a life expectancy <6 months, or severe cognitive impairment hampering communication with the physician. The study was approved by the institutional Ethics Committee and conducted in accordance with the Helsinki Declaration and Good Clinical Practice guidelines. Written informed consent was obtained from all patients.

2.2. Clinical assessment

Complete demographic and clinical details were collected at baseline for each patient (Table 1). At the first visit, both cases and controls underwent traditional comprehensive geriatric assessment (CGA)

Table 1
Demographic and clinical characteristics of cancer patients and controls.

	All patients, *n* (%)	Cancer patients, *n* (%)	Controls, *n* (%)
Age (yrs)			
n	91 (100)	52 (57.2)	39 (42.8)
Median, range	81, 70–92	81, 72–92	80, 70–91
Gender			
Male	26 (28.6)	19 (36.5)	7 (17.9)
Female	65 (71.4)	33 (63.5)	32 (82.1)
Performance status (ECOG)			
0–1	83 (91.2)	51 (98.1)	32 (82.1)
≥2	8 (8.8)	1 (1.9)	7 (17.9)
Social condition			
Home	90 (98.9)	51 (98.1)	39 (100)
Nursing home	1 (1.1)	1 (1.9)	0 (0.0)
Type of assistance			
Alone	23 (25.3)	9 (17.3)	14 (35.9)
Family	59 (64.8)	38 (73.1)	21 (53.8)
Others	9 (9.9)	5 (9.6)	4 (10.3)
Caregiver assistance (hours/day)			
Median time, range	24, 2–24	24, 2–24	24, 3–24
Tumor stage (TNM)			
I	–	16 (30.8)	–
II	–	33 (63.5)	–
III	–	3 (5.7)	–
Modality of diagnosis			
Symptoms/self examination	–	40 (76.9)	–
Screening	–	9 (17.3)	–
Incidental diagnosis	–	3 (5.8)	–
Therapeutical choice			
Adjuvant therapy as for younger adults	–	31 (59.6)	–
Adapted treatment	–	18 (34.6)	–
No adjuvant therapy	–	3 (5.8)	–
Agreement to proposed therapy			
Ready	–	51 (98.1)	–
Patient refusal	–	1 (1.9)	–
Cause of admission to hospital/ outpatient services			
Cardiovascular disease	–	–	18 (46.2)
Peripheral deep venous thrombosis	–	–	4 (10.2)
Gastrointestinal inflammatory disorders or bleeding	–	–	5 (12.8)
Screening of osteoporosis with no active comorbidity	–	–	12 (30.8)

administered by a multidisciplinary team, including a medical oncologist, a geriatrician and a psychologist. As reported elsewhere (Basso and Monfardini, 2004), the CGA included Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), Short Portable Mental Status Questionnaire (SPMSQ), and the Mini Mental Status Examination (MMSE). Comorbidities and their severity were studied according to the Cumulative Illness Rating Scale-Comorbidity Index (CIRS-CI) and Cumulative Illness Rating Scale-Severity Index (CIRS-SI) (Conwell et al., 1993). Affective status was assessed with the Geriatric Depression Scale (GDS) (Yesavage et al., 1982–1983). Nutritional status was explored with the Mini Nutritional Assessment (MNA) (Guigoz and Vellas, 1999). Social aspects included household composition, institutionalization, and amount of assistance provided by a care-giver. The number of drugs taken by patients for concomitant diseases was also recorded. The CGA results were interpreted as previously reported by Balducci (2003), classifying patients as fit, vulnerable or frail. A multidimensional prognostic index (MPI) score was also calculated based on findings for the ADL, IADL, SPMSQ, CIRS-CI, CIRS-SI, MNA, number of drugs and social conditions, and assuming a low, moderate or high mortality risk for MPI $\leq$0.33, 0.34–0.66, and >0.66, respectively (Giantin et al., 2013; Pilotto et al., 2008).

2.3. Biomarker analyses

Peripheral blood samples were collected at the time of enrollment, prior to any oncological medical treatment (endocrine therapy, chemotherapy, radiotherapy or immune therapy). Samples were analyzed for the standard blood parameters (Table 1S, Supplementary data), for Cytomegalovirus (CMV) status (Table 1S, Supplementary data) and for the following tests.

2.4. Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood by centrifugation on a Ficoll-Paque gradient (Freguja et al., 2011). Cells were stained with the following labeled monoclonal antibodies (mAbs): anti-CD3 (FITC), anti-CD4 (PerCP), anti-CD8 (PerCP), anti-CD31 (PE), anti-CD27 (PE) and anti-CD45RA (APC). Appropriate isotypic controls (mouse IgG1-PE and mouse IgG2b-APC) were used to assess non-specific staining. All samples were analyzed by four-color flow cytometry on a fluorescence-activated cell sorter (FACS) Calibur (Becton-Dickinson) (Freguja et al., 2011). Data were processed with CellQuest Pro Software (Becton-Dickinson) and analyzed with Kaluza® Analysis Software v.1.2 (Beckman Coulter, Inc.). The percentage of $CD45RA^+CD27^+$, $CD45RA^-CD27^-$ and $CD45RA^+CD31^+$ cells was calculated within the $CD3^+CD4^+$ or $CD3^+CD8^+$ gate. $CD45RA^+CD27^+$ cells were defined as naïve, $CD45RA^-CD27^-$ cells as effector memory (Anselmi et al., 2007) and $CD45RA^+CD31^+$ cells as RTE (Kimmig et al., 2002).

2.5. TREC quantification

Thymic output in PBMC was studied by measuring TREC levels by real-time polymerase chain reaction (PCR), exactly as described previously (De Rossi et al., 2002; Ometto et al., 2002). TREC levels were expressed as the number of TREC copies per 10^5 PBMC (Anselmi et al., 2007; De Rossi et al., 2002).

2.6. Telomere length measurement

Telomere length in PBMC was determined by real-time PCR exactly as described elsewhere (Rampazzo et al., 2010, 2012), and values were expressed as relative telomere/single-copy gene (T/S) ratio (Rampazzo et al., 2010).

2.7. Telomerase activity quantification

Telomerase activity was quantified by real-time PCR, as described elsewhere (Rampazzo et al., 2012) and expressed in relative units (RU).

2.8. Statistical analysis

Given the exploratory nature of the present study, a target sample size of 60 patients (30 patients per group) was calculated according to Browe (1995). The enrolment target was increased to 90 to ensure an adequate sample size should some samples be excluded due to laboratory-related problems.

Differences in geriatric parameters between cancer patients and controls were examined with the Mann–Whitney *U* test for non-parametric data and Pearson's χ^2 test with odds ratios (OR) and 95% confidence intervals (CI) for nominal data (CGA, MPI, ADL, IADL). The Mann–Whitney *U* test was used to compare subsets of lymphocytes, TREC levels, telomerase activity and CMV serology in cancer patients and controls. Student's t-test was used to compare the telomere lengths. Correlations between age and TREC levels, or telomere length in both groups were analyzed with Pearson's χ^2 test. Spearman's rank correlation was used to analyze associations between geriatric parameters and TREC levels, telomere lengths or telomerase activity. TREC levels and telomere length were also analyzed as dichotomous variables (cut-off: $\leq$median) and OR were estimated with a logistic regression model. All statistical analyses were performed with SPSS Software, Version 18 (SPSS Inc.). All p-values were two-tailed, and were considered significant when lower than 0.05.

3. Results

3.1. Characteristics of the study population

Ninety-one patients, 52 with cancer (26 breast and 26 colorectal cancer) and 39 controls, were enrolled in the study. Their demographic and clinical characteristics are listed in Table 1. There were more women in the control group (82.1%) than among the cancer patients (63.5%); the latter had better Karnofsky performance status than controls. As established by the study protocol, before enrollment, cancer patients underwent a thorough radiological assessment to rule out metastases. According to the TNM staging system, 16 patients (30.8%) had stage I, 33 (63.5%) stage II, and 3 (5.7%) stage III disease. Thirty-one patients were prescribed the same adjuvant therapy as for younger adults, according to good clinical practice, 18 patients received an adapted treatment, due to problems detected at the CGA, and 3 were given no adjuvant therapy, due to frailty detected at the CGA and were only followed up.

3.2. Comprehensive geriatric assessment

All cancer patients and controls underwent the full CGA; 17.3% of the former and 43.6% of the latter were classified as frail (OR = 0.29, 95% CI 0.10–0.82), while the proportion of vulnerable patients in the two groups did not differ significantly (Table 2). 5.8% of cancer patients and 23.1% of controls had a moderate MPI (OR = 0.20, 95% CI 0.05–0.79), and only one control had a severe MPI score. When MPI was calculated as a continuous variable, the median score was significantly lower for cancer patients than for controls (Table 2). In ADL and IADL, cancer patients tended to have preserved greater physical autonomy than controls, although the difference was not statistically significant (Table 2). Controls had better cognitive status in both the MMSE and SPMSQ, but their burden of associated diseases (as shown by CIRS-CI and CIRS-SI) was higher, as was their use of drugs for these comorbidities (Table 2).

3.3. Standard hematological, biochemical parameters and CMV status

The hematological parameters of cancer patients and controls differed only in their platelet count (Table 1S, Supplementary data). Controls had significantly lower levels of nutritional markers, such as total plasma proteins, triglycerides, and LDL-cholesterol; CRP and IL-6 were significantly higher in controls than in cancer patients (Table 1S, Supplementary data). The overall prevalence of CMV-IgG seropositive individuals was 96.7%. Antibodies against CMV were found in 49 (94.2%) of cancer patients and 39 (100%) of controls. No statistical difference was found between CMV antibody titers between the groups ($p = 0.498$) (Table 1S, Supplementary data).

3.4. Immune senescence markers

Cancer patients and controls had comparable percentages of total $CD4^+$ lymphocytes, naïve $CD4^+CD45RA^+CD27^+$ and RTE $CD4^+CD45RA^+CD31^+$ cells (Table 3). By contrast, the median (interquartile) percentages of naïve $CD8^+CD45RA^+CD27^+$ and RTE $CD8^+CD45RA^+CD31^+$ cells were significantly lower in cancer patients than in controls (16.7% [9.3–25.2] *versus* 24.6% [14.7–33.5]; $p = 0.003$) and (34.2% [24.7–46.4] *versus* 44.9% [36.0–50.7]; $p = 0.004$), respectively (Table 3). Notably, the lower percentage of $CD8^+$ naïve cells in cancer patients was compensated by a higher expansion of $CD8^+$ memory cells; thus, cancer patients and controls did not differ in terms of their percentages of total $CD8^+$ cells (Table 3). In particular, among the $CD8^+$ cell subsets, the $CD45RA^-CD27^-$ (effector memory) cells were found to be significantly higher in cancer patients than in controls (21.7% [12.7–31.7] *versus* 16.3% [10.4–25.0]; $p = 0.042$). The imbalance in favor of the CD8 memory cell subset in cancer patients was evident even when the absolute cell count was considered (not shown). For a few subjects with available frozen samples (11 cancer patients and 8 controls), markers of immune senescence ($CD28^-CD57^+$) were investigated. Cancer patients and controls exhibited similar percentages of $CD4^+CD28^-CD57^+$, but $CD8^+$ cells with senescent phenotype tended to be higher in the former ($p = 0.082$) (Table 3S, Supplementary data).

TREC levels in PBMC were significantly higher in controls than in cancer patients (25.0 [14.0–56.0] *versus* 16.0 [7.7–31.5] TREC copies/10^5 PBMC; $p = 0.031$) (Table 3). TREC levels decreased significantly with increasing age in cancer patients ($r = -0.478$; $p < 0.001$), but did not correlate with age in controls ($r = 0.071$; $p = 0.677$) (Fig. 1A).

The mean telomere length in PBMC was significantly lower in cancer patients than in controls; the former had a mean (±standard deviation (SD)) telomere length of 1.09 ± 0.31 T/S *versus* 1.22 ± 0.30 T/S in controls, $p = 0.046$ (Table 3). Telomere length correlated inversely with age in controls ($r = -0.354$, $p = 0.031$), but not in cancer patients ($r = -0.011$, $p = 0.938$) (Fig. 1B). Telomerase activity was significantly higher in cancer patients, being detected in 38/52 patients (73.1%; median value 14.6 RU) and 20/38 controls (52.6%; median value 1.4 RU); $p = 0.003$. Telomerase activity was not influenced by age in either group.

TREC was defined as high or low, and telomere length as long or short according to their values above and below the median, respectively. Subjects with TREC low/telomere short profile were at higher risk of cancer than subjects with only low TREC or short telomere (Table 4).

3.5. Relationship between TREC levels, telomere length and geriatric characteristics

In the control group, CIRS-SI was the only tool which revealed a significant positive association with TREC level ($r = 0.45$, $p = 0.01$) and telomere length ($r = 0.35$, $p = 0.03$), whereas it had a significant negative correlation with the number of drugs taken for concomitant diseases ($r = -0.39$, $p = 0.02$) (Table 2S, Supplementary data). No relationship emerged between telomere length, thymic output and geriatric features

Table 2
Scores of geriatric tools in cancer patients and controls.

Geriatric tool	All patients	Cancer patients	Controls	OR (95% CI)	p-Value
CGA, *n* (%)					
Fit	37 (40.7)	24 (46.2)	13 (33.3)	1	
Vulnerable	28 (30.7)	19 (36.5)	9 (23.1)	1.14 (0.40–3.24)	0.801[a]
Frail	26 (28.6)	9 (17.3)	17 (43.6)	0.29 (0.10–0.82)	0.020[a]
MPI, *n* (%)					
Low	78 (85.7)	49 (94.2)	29 (74.3)	1	
Moderate	12 (13.2)	3 (5.8)	9 (23.1)	0.20 (0.05–0.79)	0.022[a]
Severe	1 (1.1)	0 (0.0)	1 (2.6)	<0.01	ns[a]
MPI					
Median, range	0.25, 0.00–0.69	0.19, 0.00–0.44	0.25, 0.06-0.69	–	0.001[a]
Dependencies in ADL, *n* (%)					
No	71 (78.0)	44 (84.6)	27 (69.2)	1	
≥1	20 (22.0)	8 (15.4)	12 (30.8)	0.41 (0.15–1.14)	0.790[a]
Dependencies in IADL, *n* (%)					
No	57 (62.6)	36 (69.2)	21 (53.8)	1	
≥1	34 (37.4)	16 (30.8)	18 (46.2)	0.52 (0.22–1.23)	0.133[a]
MMSE					
Median [IQR]	27.1 [24.4–28.4]	26.2 [24.4–27.3]	28.3 [26.4–29.3]	–	0.001[b]
SPMSQ					
Median [IQR]	1.0 [0.0–2.0]	1.0 [0.0–2.0]	0.0 [0.0–2.0]	–	0.013[b]
CIRS-CI					
Median [IQR]	3.0 [1.0–4.0]	2.0 [1.0–3.0]	3.0 [2.0–4.0]	–	0.032[b]
CIRS-SI					
Median [IQR]	1.5 [1.3–1.6]	1.4 [1.2–1.5]	1.6 [1.4–1.8]	–	<0.001[b]
GDS					
Median [IQR]	3.0 [1.0–6.0]	3.0 [1.2–6.0]	3.0 [1.0–6.0]	–	0.888[b]
BMI					
Median [IQR]	25.7 [24.0–27.4]	25.8 [24.2–26.9]	25.0 [23.0–28.6]	–	0.911[b]
Drugs for concomitant diseases					
Median [IQR]	5 [3–6]	4 [2–5]	5 [4–8]	–	0.003[b]

[a] Pearson's χ^2 or Fisher's test.
[b] Mann–Whitney *U* test.

Table 3
Thymic output and telomere length in cancer patients and controls.

Parameter	All patients Median [IQR]	Cancer patients Median [IQR]	Controls Median [IQR]	p-Value
% $CD3^+$	54.3 [40.8–65.2]	53.6 [37.8–65.5]	56.3 [41.8–65.2]	0.560 [a]
% $CD4^+$	35.5 [25.6–49.1]	35.0 [21.1–46.7]	35.6 [26.6–51.8]	0.389 [a]
% $CD8^+$	15.7 [10.1–22.4]	15.4 [9.7–22.6]	16.4 [10.5–22.8]	0.717 [a]
% naïve, $CD4^+CD45RA^+CD27^+$	34.0 [22.5–48.5]	29.6 [17.9–48.3]	38.0 [27.0–49.8]	0.076 [a]
% naïve, $CD8^+CD45RA^+CD27^+$	20.5 [10.9–29.5]	16.7 [9.3–25.2]	24.6 [14.7–33.5]	0.003 [a]
% RTE, $CD4^+CD45RA^+CD31^+$	21.3 [14.2–33.4]	20.3 [10.4–32.0]	23.3 [16.8–33.5]	0.176 [a]
% RTE, $CD8^+CD45RA^+CD31^+$	37.6 [29.1–48.9]	34.2 [24.7–46.4]	44.9 [36.0–50.7]	0.004 [a]
TREC copy number/10^5 PBMC	19.0 [10.1–37.0]	16.0 [7.7–31.5]	25.0 [14.0–56.0]	0.031 [a]
Telomere length (T/S ratio)[c]	1.15 ± 0.30	1.09 ± 0.31	1.22 ± 0.30	0.046 [b]
Telomerase activity				
n positive/*n* tested	58/90	38/52	20/38	
Relative units	5.6 [0.0–26.0]	14.6 [0.0–36.8]	1.4 [0.0–6.4]	0.003[a]

[a] Mann–Whitney *U* test.
[b] Student's *t*-test.
[c] Data are expressed as mean ± SD.

of cancer patients (Table 2S, Supplementary data). Neither a classification of unfitness at CGA (not shown) nor the MPI score (Table 2S, Supplementary data) correlated significantly with thymic output or telomere length in either group. Among the controls, TREC levels correlated positively with IL-6 ($r = 0.34$, $p = 0.04$) and negatively with total plasma protein levels ($r = -0.38$, $p = 0.02$). Neither of these correlations emerged in the cancer patients (data not shown).

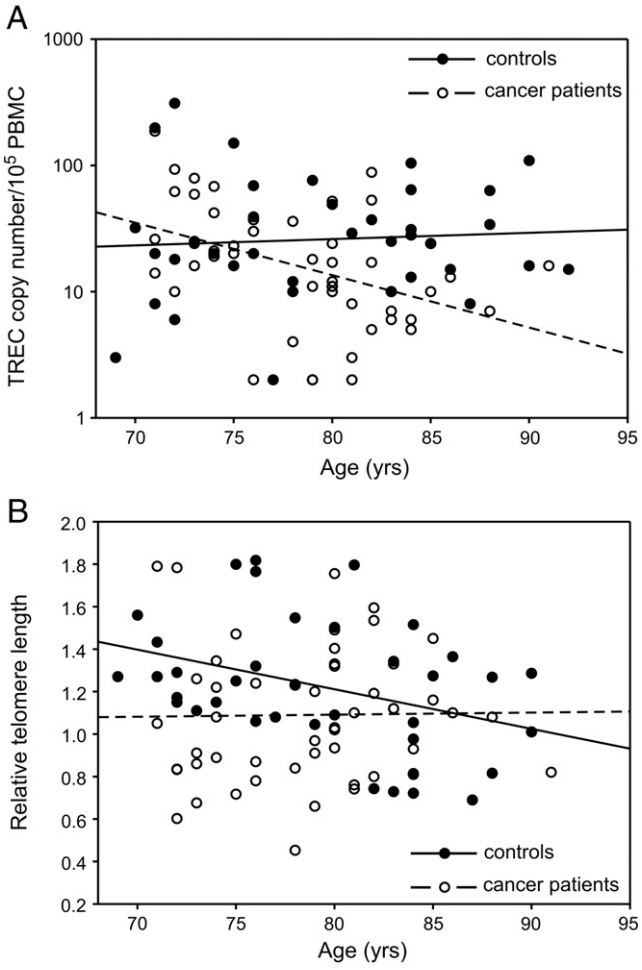

Fig. 1. Correlation between age and (A) TREC levels and (B) telomere lengths in cancer patients and controls. Panel (A): TREC levels decline with age in peripheral blood cells of cancer patients ($r = -0.478$, $p < 0.001$), but not in controls ($r = 0.071$, $p = 0.677$). Panel (B): telomere lengths decrease with age in peripheral blood cells of controls ($r = -0.354$, $p = 0.031$), but not in cancer patients ($r = -0.011$, $p = 0.938$).

4. Discussion

This is the first study aiming to shed light on two essential aspects of the aging process (thymic output and telomere length in peripheral blood cells) in elderly cancer patients. We found that 70- to 92-year-old cancer patients had significantly lower TREC levels, lower percentages of naïve and RTE $CD8^+$ T lymphocytes, and a more expanded $CD8^+$ memory cell subset than age-matched controls. These results are partially consistent with those of a small trial in which head and neck cancer patients (most of them under 70 years old) revealed lower TREC levels than controls (Kuss et al., 2005). The expansion of memory $CD8^+$ cells, particularly those with effector phenotype, is also consistent with the findings of two recent studies of breast cancer patients (Hueman et al., 2007; Poschke et al., 2012). The shift from naïve T cells to memory cells was probably due to greater stimulation by tumor antigens; as $CD8^+$ T cells are the key components of tumor immune surveillance, the tumor-induced dysfunction may be more evident in the CD8-cell subset than in the CD4-cell compartment (Klebanoff et al., 2006; Williams and Bevan, 2007).

Our findings confirm reports by other authors that thymic activity does not stop completely beyond 70 years of age (Mitchell et al., 2010; Nasi et al., 2006). Notably, while TREC levels in cancer patients dropped significantly with increasing age, they remained relatively constant in controls. Thymic output may compensate for the loss of peripheral blood lymphocytes in elderly patients, but this homeostatic phenomenon

Table 4
TREC and telomere profile on cancer patients and controls.

	Cancer patients *n* (%)	Controls *n* (%)	OR (95% CI)	p-Value
TREC levels[a]				
TREC high	20 (40.0)	23 (62.2)	1	
TREC low	30 (60.0)	14 (37.8)	2.46 (1.03–5.90)	0.043
Telomere length[b]				
T/S long	21 (40.4)	23 (62.2)	1	
T/S short	31 (59.6)	14 (37.8)	2.43 (1.02–5.76)	0.045
TREC levels & telomere length				
Others[c]	31 (62.0)	30 (85.7)	1	
TREC low & T/S short	19 (38.0)	5 (14.3)	3.68 (1.22–11.11)	0.021

[a] TREC low: TREC ≤ median, TREC high: TREC > median.
[b] T/S short: T/S ≤ median, T/S long: T/S > median (the median value is coincident with the mean value).
[c] Others: TREC high & T/S long, TREC low & T/S long and TREC high & T/S short.

seems to disappear in cancer patients. In our study, the control group included a higher proportion of frail patients, with higher average MPI scores and more chronic diseases and comorbidities than the cancer patients. Levels of pro-inflammatory cytokine IL-6 were also significantly higher in controls than in cancer patients. Systemic inflammation and loss of peripheral blood cells may stimulate thymic output, which plays an important role in immunological homeostasis. In fact, TREC levels did correlate significantly with CIRS-CI in controls, but not in cancer patients. The imbalance between the two groups in terms of their geriatric conditions reinforces the magnitude of the difference observed for TREC levels between cancer patients and controls. While the hypothesis of immune homeostasis may justify a higher thymic output in controls, the lower age-related TREC levels seen in cancer patients may point to a pre-existing condition, favoring immune escape and the onset of malignant disease.

In agreement with the lower percentage of $CD8^+$ naive and RTE cells in cancer patients than in controls, the former tended to have a higher percentage of $CD8^+$ immune senescent cells. Several data indicate that latent CMV infection leads to significant changes in the $CD8^+$ repertoire, and it may be an important driver of immune senescence (Almanzar et al., 2005; Solana et al., 2012). The above results are unlikely to be due to CMV infection, because all except three cancer patients were CMV-positive and with similar titers of anti-CMV antibodies.

As regards telomere length, there are many data available on the relationship between telomere length and aging-associated changes and chronic diseases (Aviv, 2006; Baird, 2006; Wong and Collins, 2003), but data are lacking for elderly cancer patients. Our study showed that cancer patients' telomeres in PBMC were significantly shorter than those seen in controls. In addition, while control patients' telomeres became shorter with age, as expected according to previous studies (Blasco, 2005; Der et al., 2012; Steenstrup et al., 2013), no such relationship between age and telomere length was seen in our cancer patients. This difference cannot be explained by lower telomerase activity, because it was detected in the PBMC of most cancer patients. The fact that controls scored worse on the severity and comorbidity indexes than cancer patients clearly highlights the magnitude of the difference in telomere length between the groups.

Several studies have found shorter telomeres in tumor cells than in surrounding non-cancer cells (Bisoffi et al., 2006; Rampazzo et al., 2010), but the intriguing finding of shorter telomeres in PBMC cannot be explained by the presence of cancer cells in peripheral blood. Our cancer patients had radically resected early breast or colorectal cancer, and had undergone thorough radiological assessment to exclude both persistent local disease and distant metastases. As reported elsewhere (Franken et al., 2012; Shimada et al., 2012), the number of epithelial cells detectable in the peripheral blood of patients with early breast or colorectal cancer is so small that it cannot influence telomere length or telomerase activity. We surmised that our control patients' longer telomeres were related to their higher proportion of naïve $CD8^+$ cells. To explore this issue, we estimated telomere length separately in $CD8^+$ naïve and $CD8^+$ memory cells from three cancer patients: the two subsets had a similar telomere length, indicating that the shorter telomeres cannot be explained by an excessive replication/expansion of the memory cell subset (not shown). We therefore suggest that telomere length in peripheral blood cells reflects the more advanced biological aging of the immune system in cancer patients than in controls, a pre-existing condition which facilitates the onset of cancer in the former.

Neither thymic output nor telomere length correlated with the results of the CGA or MPI scores in our study population. These results are consistent with studies in elderly-non cancer patients, which found no association between frailty indexes and telomere length (Collerton et al., 2012; Woo et al., 2008). Therefore, the relationship between these markers and immune senescence and aging cannot be extrapolated to the functional level represented by the geriatric assessment of frailty.

5. Conclusions

This exploratory study is the first to find that elderly cancer patients have significantly lower thymic output and shorter telomeres in their peripheral blood cells than age-matched non-cancer patients; these results indicate that immune senescence may hamper the ability of the immune system to prevent cancer initiation and/or control of tumor progression. Overall, these findings suggest the hypothesis, which would need to be tested in a larger study, that elderly people with shorter telomeres and lower thymic output are at higher risk of developing cancer.

Conflict of interest

None of the authors have any conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2013.09.011.

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